Mechanisms of Muscle Injury after Eccentric Contraction

Richard L Lieber¹ & Jan Fridén²

¹Departments of Orthopaedics and Bioengineering, Biomedical Sciences Graduate Group, University of California and Veterans Administration Medical Centers, San Diego, CA USA
²Department of Hand Surgery, Göteborg University, Sweden


Eccentric contractions of skeletal muscles produce injury and, ultimately, muscle strengthening. Current data suggests that the earliest events associated with injury are mechanical in nature and may be based primarily on the sarcomere strain experienced by the muscle. In this review, recent experimental data, primarily from rabbit dorsiflexor muscles, are used to provide general information regarding the factors that cause injury and means for preventing injury. Mechanical experiments reveal that excessive sarcomere strain is the primary cause of injury. We hypothesize that excessive strain permits extracellular or intracellular membrane disruption that may permit hydrolysis of structural proteins leading to the myofibrillar disruption that is commonly observed. Inflammation that occurs after injury actually further degrades the tissue, but prevention of the inflammation leads to a long-term loss in muscle function. Simple treatments such as increasing muscle oxidative capacity ("getting into shape") or cyclic stress-relaxation of tissue ("stretching out") have no measurable effect on the magnitude of muscle injury that occurs. Ultimately, an improved understanding of the damage mechanism may improve our ability to provide rehabilitative and strengthening prescriptions that have a rational scientific basis.

Introduction

Athletic and fitness programs are on the rise throughout the developed world. It is estimated that several hundred million individuals are involved in organized sports and regular exercise. This increased emphasis on fitness has led to a dramatic increase in the number and type of activity-related injuries. In fact, over 20 million American people per year sustain injuries severe enough to limit activity in the workplace. Athletic activity in the form of rehabilitation is also on the rise both in the athletic and elderly population to expedite recovery after injury or hospitalization. In both cases, rapid muscle strengthening and functional restoration are the goal.

New textbooks and a recent symposium on the subject (Clarkson, 1992) emphasize the two-edged muscle response to eccentric contraction (EC): on the positive side, EC provides a potent muscle strengthening stimulus; on the negative side, EC is specifically associated with muscle injury and soreness. This correlation has led many to state, unequivocally, that injury is required for muscle strengthening. Stated in the vernacular of the strength training enthusiast, "You have to break it down to build it up!" Several investigators have reported that
eccentric training provides a protective effect against further EC-induced muscle injury. Unfortunately, we lack basic information regarding the cellular mechanisms of EC-induced muscle injury and training that would provide a rational basis for injury prevention, strength training and rehabilitation. The purpose of this review is to provide information from experiments using animal models that improve our understanding of the mechanism of eccentric contraction-induced injury as well as studies of injury prevention.

**A Physiological Method of EC-induced Injury and Strengthening was developed**

To mimic the cyclic activation and length changes of human muscle subjected to intense exercise, we connected the distal tendon of the rabbit tibialis anterior muscle directly to a dual-mode servo motor (Fig. 1) enabling simultaneous muscle lengthening and force measurement (Lieber & Fridén, 1989). We were initially concerned that our animal model accurately represent the human situation. Thus, we “calibrated” our injury model by comparing ultrastructural changes observed in rabbit tibialis anterior (TA) muscles with those seen in human vastus lateralis muscles (Fridén, Sjöström & Ekblom, 1983). Using a computer-controlled data acquisition system (Lieber, Smith, Campbell & Hargens, 1986), we actively lengthened muscles at a magnitude and rate that mimics that seen in the ankle flexors during rapid running (Goslow, Reinking & Stuart, 1973; Dimery, 1985). We demonstrated that the injury associated with eccentric contraction shown to be characteristic of human muscle (disruptions of the Z-line and myofibrillar apparatus), was also observed after eccentric contraction in the animal model system (Fig. 2).

![Figure 1](image-url)  
*Figure 1: Experimental method for invasively forcing eccentric contractions upon the rabbit tibialis anterior muscle and measuring contractile properties. The distal tendon is attached to a dual-mode motor. Muscle stimulation is accomplished via a nerve cuff placed around the peroneal nerve. The entire process is computer-controlled. (From Lieber et al., 1991).*
Rabbit Muscle Architecture was measured to Predict Fiber Strain during EC

Since skeletal muscle fibers do not usually run the entire muscle length, choosing the muscle deformation magnitude was not a straightforward problem. Many previous experiments had stretched muscles and created “muscle injury”. However, deformation magnitudes were expressed relative to the whole muscle length not muscle fiber length. Thus, early results were confusing in that identical deformations applied to muscles of identical length resulted in very different degrees of damage. We thus performed a systematic study of the orientation, length, and number of muscle fibers in the various rabbit hindlimb muscles (Lieber & Blevins, 1989). Interestingly, we found that the design of the different hindlimb muscles varied widely between functional groups (i.e., plantarflexors, dorsiflexors, quadriceps and hamstrings). Such specialization is analogous to the various gears in the car—muscles are designed to provide either large excursions or large forces depending on the architectural arrangement of fibers. These architectural data proved invaluable in understanding the differential muscle injury that we subsequently observed in the different ankle flexors.

EC caused greater Injury than Isometric Contraction or Passive Stretch

We initiated a study of eccentric contraction-induced injury where strain (expressed relative to muscle fiber length, $L_f$ based on architectural measurements) and strain rate were constrained within the physiological range. The magnitude of the stretch was 25% of the TA $L_f$ (determined individually for each muscle) and the strain rate was 125%/sec. Using the apparatus shown in Fig. 1, three treatments were imposed upon skeletal muscles (Lieber, McKee-Woodburn & Fridén, 1991): passive stretch, isometric contraction, or stretch superimposed on contraction yielding the EC.
Cyclic activation (in the cases of isometric and eccentric contraction) and stretch (in the cases of eccentric contraction and passive stretch) lasting 400 ms were imposed upon the muscle every 2 seconds for thirty minutes. Then, muscle contractile properties were again measured to provide a measurement of muscle injury and fatigue. In all cases, muscle strength ($P_o$) decreased following treatment (Fig. 3). However, the magnitude of the force deficit was a strong function of the treatment method. Thus, the magnitude of force decline following eccentric contraction was significantly greater than that observed following either cyclic isometric contraction or cyclic passive stretch. This provided evidence that, indeed, something unique occurred in the muscle after eccentric contraction.

**Abnormally Enlarged Fibers were only of the Fast Glycolytic (FG) Fiber Type**

To understand the basis for the contractile results obtained, we examined exercised muscles at both the light and electron microscopic levels. While no ultrastructural abnormalities were observed in any of the muscles from either the isometric or passive stretch groups, a significant portion of the fibers in the EC group displayed various degrees of disorganization of the sarcomeric banding pattern such as we had observed in human eccentric exercise. Streaming of the Z-disk material, focal loss of Z-disks, and extension of Z-disks into adjacent A-bands were commonly seen (Fig. 2).

Similarly, while the light microscopic morphology of samples from both the passive stretch and isometric contraction groups was normal, a dramatic abnormality was found in eccentrically exercised muscles wherein greatly enlarged fibers were seen in cross-section (Fig. 4). These fibers appeared rounded, more lightly stained by hematoxylin and eosin, and 3-4 times the normal. Only fibers from muscles in the eccentric contraction group demonstrated this abnormal appearance, and they were always depleted of glycogen, confirming that they had been activated. (When a muscle fiber is repetitively activated, its
intracellular glycogen store is depleted, serving as a morphological marker for activation.)

Since muscle fiber types are heterogeneous with respect to contractile speed and metabolism, we determined which fiber type was damaged in order to provide insights into the damage mechanism (Lieber et al., 1991). Fiber type was determined in serial sections and we found that all enlarged fibers were exclusively of the fast glycolytic (FG) fiber type! These enlarged fibers demonstrated dramatic size variation even in serial sections (Fridén & Lieber, 1998). This finding of fiber type specific injury allowed us to delve more deeply into damage mechanisms by identifying features unique to FG fibers that could render them more vulnerable compared to other fibers. For example, since FG fibers have low oxidative capacity, this suggested that oxidative capacity was important in determining the extent of fiber damage. Implicating oxidative capacity in muscle injury is appealing for several reasons. First, it explains the well-known "protective" effect of endurance training on EC-induced damage (Evans, Meredith, Cannon, Dinarello, Frontera, Hughes, Jones & Knuttgen, 1985) since endurance training is known to result in increased muscle oxidative capacity (Gollnick, Armstrong, Saubert, Piehl & Saltin, 1972), and, therefore, FG to FOG fiber subtype conversion. Oxidative capacity importance was also supported by our observation that FG fibers are selectively damaged after tourniquet ischemia (Lieber, Pedowitz, Friden & Gershuni, 1992). Recent reports also demonstrated that FG fibers are also especially vulnerable to ischemia (Caiozzo, Gardner, Starr, Najarian & Prietto, 1990; Lieber et al., 1991; Suematsu, DeLano, Poole, Engler, Miyasaka, Zweifach & Schmid-Schönbein, 1994). However, experiments in which muscle oxidative capacity was artificially increased by electrical stimulation (see
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below) failed to support our hypothesis that low oxidative capacity predisposes a muscle fiber to injury. We still believe that understanding the mechanism of selective damage to FG fiber types will lead to a better understanding of muscle injury in general.

Muscle Injury resulted from High Fiber Strain

Having established that eccentric contraction resulted in fiber type-specific muscle injury and decreased performance, the question remained: What are the mechanics of the injury itself? Several mechanisms had been proposed (Evans & Cannon, 1991) including direct mechanical injury, ischaemic injury, metabolic injury and injury resulting from an inability to adequately buffer calcium. We first pursued the mechanical mechanism.

The fact that eccentric contractions are associated with high force and result in muscle damage had historically led to the presumption that the high muscle forces caused the muscle injury. While this was attractive in theory there was only anecdotal experimental evidence in support of such a claim. We therefore designed an experiment in which muscle stress and strain were systematically altered and muscle strength measured after cyclic eccentric contraction at different stresses and strains (Lieber & Fridén, 1993). Specifically, stimulation timing was altered relative to the muscle deformation in order to achieve altered stresses at identical strains (Fig. 5) at two different strain magnitudes. These data were analyzed using a stepwise regression model and we found that the magnitude of injury was closely related to the magnitude of the muscle strain as opposed to the stress imposed on the fibers (Lieber & Fridén, 1993). Interestingly, a recent report presented the opposite result for predominantly slow-contracting soleus muscles (Warren, Hayes, Lowe & Armstrong, 1993) which may be due to the different muscles used or the different protocols designed to impose different stresses and strains upon the different muscles.

Figure 5: Experimental design to vary stress and strain for determination of muscle injury mechanism. Lower panel: muscle deformation in terms of muscle fiber length (L0). Upper panel: muscle tension. Horizontal stippled bar represents the stimulation period. Vertical dotted lines represents the onset of muscle length change.

(A) Low stress contraction (B) High stress contraction at same strain as (A). (Data from Lieber & Fridén, 1993).
Muscle Fiber Length may explain Selective FG Fiber Damage

A potential explanation for selective FG fiber injury can be synthesized from recent reports on muscle fiber orientation within whole muscles. Muscle fibers belonging to different motor unit types may have different lengths based on the report of Loeb, Pratt, Chanard & Richmond (1987), who showed that muscle fibers did not simply extend from one tendon plate to the other but were actually arranged in series along the length of the muscle. Dr. Reggie Edgerton’s laboratory (Ounjian, Roy, Eldred, Garfinkel, Payne, Armstrong, Toga & Edgerton, 1991) extended this observation by measuring fiber length directly in different cat tibialis anterior motor unit types using the glycogen depletion method. Surprisingly, they found that many FF motor units (which contain FG fibers) actually ended or even originated within the muscle belly itself! This obviously raised a number of questions regarding force generation in muscle which remain to be answered. However, these observations suggest that muscle deformation may be differentially distributed between muscle fiber types so that strain experienced by fiber types is not equivalent. This assertion was supported by physiological experiments by Huijing & Baan (1992), who, by selective motor unit activation, measured systematically different length-tension properties of low threshold (presumably type SI) and high threshold (presumably type FF) motor units. Taken together, these studies support the assertion that differential FG fiber type injury may have a simple geometrical explanation, namely, that FG fibers experience increased strain and injury due to their short fiber length.

Electrical Stimulation Training does not Protect Muscles against EC-Induced Injury

An intervention used in an attempt to decrease muscle injury was isometric electrical stimulation delivered at a frequency of 10 Hz. Recall that one of our earliest observations was selective damage to the FG muscle fiber. Since FG fiber oxidative capacity is extremely low and since training can dramatically increase FG fiber oxidative capacity, actually converting it to the FOG fiber type, (Lieber, 1986c; Lieber, 1986a; Lieber, 1986b), we hypothesized that the protective effect of training was to increase fiber oxidative capacity. This hypothesis was directly tested by pre-treating rabbit anterior compartment muscles with electrical stimulation for 30 minutes/day x 5 days/week x 3 weeks. (Pilot experiments carried out for 1 to 4 weeks of stimulation revealed a plateau in the stimulation effect after 3 weeks.)

To document muscle changes due to electrical stimulation muscle fiber oxidative enzyme activity (as indicated by the citrate synthase enzyme) and muscle capillary density and geometry were measured. We found a significant increase in EDL oxidative activity (from 15.6±1.2 to 26.1±1.1 µmole/mg/min) and capillary density (from 839±56 cap/µm2 to 1026±71 cap/µm2) with smaller changes in the TA (Patel, Cuizon, Mathieu-Costello, Fridén & Lieber, 1998). Yet, in spite of these increases in the muscle’s ability to deliver oxygen and utilize it within the cell, there was no significant correlation between oxidative capacity and maximum tetanic tension for either the TA or EDL and maximum tetanic tension was not altered after injury preceded by electrical stimulation training (Fig. 6). Therefore, low oxidative capacity did not provide the explanation for selective FG fiber damage.
Figure 6: Maximum dorsiflexion torque as a function of stimulation frequency measured three days after eccentric exercise in groups subjected to electrical stimulation training prior to eccentric contraction (filled circles) or eccentric contraction without prior training (open circles). (B) TA and EDL maximum tetanic tension measured three days after eccentric exercise in groups subjected to electrical stimulation training prior to eccentric contraction (filled bars) or eccentric contraction without prior training (open bars). Stippled bars above bar graphs represent mean ± SEM of normal TA or EDL muscle. (Data from Patel et al., 1998.)

**NSAID Administration provides a Short-term Benefit but Long-Term Loss in Muscle Function**

Muscle fiber disruption, of the kind occurring after eccentric exercise, would be expected to provide a significant inflammatory stimulus. In addition, the continued tension decrease following the initial tension drop suggested that an inflammatory process may be involved after the initial injury. However, since the inflammation process (which includes proteolysis by infiltrating neutrophils and macrophages) can itself cause damage in excess of that originally experienced by the tissue, it could be argued that prevention of inflammation would improve muscle status following injury. Such a hypothesis is difficult to test in humans since the analgesic effect of nonsteroidal antiinflammatory drugs (NSAIDs) may itself permit improved performance. Thus, we quantified the effect of NSAID treatment on our rabbit skeletal muscle model following eccentric contraction-induced muscle injury. After muscle injury, one group was treated with...
flurbiprofen at a dosage of approximately 9 mg/day. The remaining animals were permitted normal cage activity as untreated controls. The duration of drug treatment was 3 days for the 3 day group, and 7 days for the 7 day group. For the 28 day group, flurbiprofen was administered only for the first 7 days post exercise. The NSAID-treated group demonstrated remarkable recovery compared to nontreated, eccentrically exercised muscles after only 3 days and 7 days, but then showed a significant decline in torque generation after 28 days (Fig. 7). This was obvious in the torque records and the force records of both the EDL and TA. This represents a short-term benefit but long term detriment of NSAID treatment. In support of this treatment-dependent effect, two-way analysis of variance revealed a highly-significant interaction between treatment and time (p<0.01). Thus, flurbiprofen-treated muscles demonstrated increased muscle strength at the early time periods but depressed strength after 28 days (Mishra, Fridén, Schmitz & Lieber, 1995).

**Desmin Loss occurs very Rapidly after Eccentric Exercise**

The most significant structural abnormality that we have observed after EC is the selective loss of the intermediate filament protein, desmin. Desmin acts as an extrasarcomeric mechanical stabilizer of myofibrillar regularity and integrity (Lazarides, 1980; Price, 1991) and, interestingly, is more abundant in slow muscle fibers than in either type FOG or type FG fibers (Boudrieau, Vincent, Côté & Rogers, 1993). We showed that desmin loss after EC is extremely rapid - it can happen as early as 5 minutes into the EC exercise bout (Lieber, Thornell & Fridén, 1996)! This is the *earliest* documented structural change observed in muscle after EC. This dramatic and rapid desmin loss, which does not occur after either isometric or concentric contraction, points to some type of enzymatic hydrolysis.
as a likely mechanism rather than gene regulation which requires much more
time. An attractive candidate for the proteolytic mechanism is the calcium
activated protease, calpain, which is present in skeletal muscle (Dayton, Goll,
Zeece, Robson & Revilla, 1976) and for which desmin is a substrate (Belcastro,
1993). The mechanism of action of calpain requires raised intracellular calcium
ion [Ca\(^{2+}\)] concentration.

**Proposed Mechanism of Eccentric Contraction-induced
Muscle Injury**

While there is, as yet, no direct evidence for such an increase, Duan et al. (Duan,
Delp, Hayes, Delp & Armstrong, 1990) demonstrated an increase in the
mitochondrial calcium concentration in muscles subjected to an exercise protocol
biased toward eccentric contraction. Since mitochondrial calcium concentration
indirectly reflects cytoplasmic [Ca\(^{2+}\)], the results of Duan et al. might be construed
as indirect evidence for increased [Ca\(^{2+}\)]. This observation, in conjunction with
our earlier demonstration that muscle fiber strain was the mechanical factor that
most strongly influenced the magnitude of muscle injury (Lieber & Fridén, 1993)
has lead to the following hypothesis regarding the early mechanism of eccentric
contraction induced muscle damage (Fig. 8): (a) muscle fiber strain results in an
increased [Ca2+]. Such an increase may be due to calcium influx via strain
activated channels (Guharay & Sachs, 1984), by disruption of the intracellular
stores of calcium in the sarcoplasmic reticulum, or by disruption of the T-system
or sarcolemma (Fig. 8A). This may be related to the concept of sarcomere
“popping” which has been proposed as a damage mechanism during eccentric
contraction (Morgan, 1990). (b) Following the increased [Ca2+], calpain activation
results in selective hydrolysis or disruption of the intermediate filament network
(Fig. 8B). It has been demonstrated that desmin is a substrate for calpain while
actin and myosin are not (Reddy, Ellinger, Rabinowitz, Fischman & Zak, 1975).
This could explain the loss of desmin in sections that still demonstrate regular
arrangements of contractile and metabolic proteins. (c) Finally, after the
intermediate filament network has been altered due to proteolysis or con-
formational changes, the myofilibrillar apparatus is disrupted on repeated muscle
activation and unable to develop normal tension (Fig. 8C). Of course, numerous
variations to this scheme could be proposed. For example, mechanical events
could alter intermediate filament structure which would then render them
vulnerable to calpain-mediated digestion or sarcolemmal integrity loss could
trigger the entire sequence.

An alternate mechanism for desmin disruption could be that the primary injury
occurs to the sarcolemma and results in the sequence of events presented above.
Experimental evidence for primary sarcolemmal injury has been presented in rat
skeletal muscle subjected to 90 minutes of eccentric exercise by downhill running
(McNeil & Khakée, 1992; Clarke, Khakée & McNeil, 1993). These authors used
immunohistochemical identification of serum albumin within the myofibers as
evidence for transient disruption of the sarcolemma. They found that, after 90
minutes of downhill running, a significant number of muscle fibers were labeled,
indicating membrane disruption. Similar results were obtained from normal
aortic endothelial cells (Yu & McNeil, 1992). It is thus possible that transient
change in sarcolemmal permeability represents a normal but heretofore
unappreciated mechanism by which muscles meet the requirements for
increasing mechanical performance or release intracellular growth factors to the
extracellular matrix.
Figure 8: Schematic depiction of eccentric contraction-induced muscle damage. Calcium ions are represented as filled dots. (A) Muscle fiber strain results in an increased Ca\(^{2+}\). (B) Increased Ca\(^{2+}\) leads to calpain activation and selective hydrolysis or disruption of the intermediate filament network. (C) After intermediate filament network damage, the myofibrillar apparatus is disrupted on repeated muscle activation. (From Lieber et al., 1996).

**Muscle Injury and Repair may be Related to Sarcomere Number**

Muscle biophysicists agree that the basic mechanism of force generation during eccentric contractions is poorly understood. For example, it is not clear why a single muscle fiber which is slowly stretched at sarcomere lengths above the optimum increases muscle force when the cross-bridge theory predicts decreased force. Similarly, there is still no completely satisfactory explanation regarding force enhancement in single muscle fibers after rapid stretch (Edman, Elzinga & Noble, 1978). Recently, Morgan proposed a theory for force generation during EC which is based on the fact that different sarcomeres along the length of a muscle, which have nonuniform sarcomere lengths, have different intrinsic strengths (Morgan, 1990). Thus, he claims that during EC, sarcomeres “pop” to very long lengths resulting in the unique force patterns observed and the injury patterns observed ultrastructurally. Experimental support for this theory has been forthcoming.

**Summary**

Eccentric contractions of skeletal muscles produce injury and, ultimately, muscle strengthening. Current data suggests that the earliest events associated with injury are mechanical in nature and may be based primarily on the sarcomere strain experienced by the muscle. After excessive sarcomere strain occurs,
extracellular or intracellular membranous disruption may permit hydrolysis of
structural proteins that leads to the myofibrillar disruption commonly observed.
In any event, an improved understanding of the damage mechanism may improve
our ability to provide rehabilitative and strengthening prescriptions that have a
solid scientific basis.

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